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Review

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Present and Future of the Radio-Molecules used in Detection of Sentinel Lymphatic Nodes

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SUMMARY. Detection of the sentinel lymph node has become a mainstay of certain surgical interventions for cancer. In this sense, physiological and biochemical properties of the molecules employed for early detection of such metastasis have become relevant in many specialties. Traditionally the main characteristics considered for such radiopharmaceuticals are particle size and surface features. In addition, design of radiolabeled molecules has to take in account anatomy of the vascular lymphatic epithelium and its interaction with such agents. The aim is to create more specific and effective drugs, thus precisely shaping and eventually remodelling the surgical strategy for a given patient. Advances in diagnostic imaging open perspectives for additional categories of agents, endowed with different physico-chemical features as required by positron-emission tomography and other sophisticated procedures. This review covers the molecules used in sentinel node finding as well as some related topics, which can help to understand their action mechanism and failures.

INTRODUCTION

Comparatively frequent types of human cancer comprise at least some 300 different diseases [1]. Lung and colorectal malignancies, along with gender-specific breast and prostate lesions, represent true public-health problems [2]. There are two basic ways for the progress of solid tumors beyond the confines of its tissue compartment. One is contiguous invasion and the other one is metastasis, by which cancer disseminates to distant organs. Generally, organs that are prone to metastasis are endowed with a rich microcapillary net, such as liver, lungs and axial bone [2]. For adenomas and carcinomas, metastasis via the lymphatic system tends to emerge before vascular new growths [3]. The concept of sentinel lymph node (SLN) was proposed by Morton *et al.* [4] in 1992, as the first node to receive drainage from a primary tumor. In principle, if this structure is tumor-free, then the lesion has good prognosis and lymphadenectomy is superfluous. Obviously the opposite should be admitted if neoplastic contamination is demonstrated. This concept has found high applicability in melanoma and breast cancer [5,6].

The aims of this review are to discuss principal radiolabeled markers used in nuclear medicine for the detection of SLN, their advantages and limitations, as well as envisage future improvements to enhance specificity, efficiency and overall diagnostic value.

ANATOMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF LYMPHATIC VESSELS

In the lymphatic system there are five main categories of channels and reservoirs: capillaries, collectors, nodes, trunks and ducts [7]. The hemodynamic role is to complement the circulatory activity of the blood vessels by means of regulation of fluid balance in the tissues, facilitating interstitial protein transportation and carrying back residual tissue fluid. Within this capacity it behaves as a gateway for leukocytes, but potentially also for tumor cells.

KEY WORDS: Cancer, Melanoma, Metastasis, Radiopharmaceutical, Sentinel Lymph Node

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Fluids and macromolecules emerging from blood capillaries are collected in the interstitial space by lymphatic capillaries and then returned to blood circulation through the body-wide net of ducts. At the same time they lead migrated lymph cells and other white cells along with antigens from tissues to the lymph nodes, and are essential in the initiation of many immune phenomena [8]. Lymphatic capillaries are composed of a single layer of unfenestrated endothelial cells, which is optimally suited for driving fluids, cells and macromolecules to the inside [9]. Lymphatic capillary lumens are more irregular and wider than blood capillaries. A unique aspect of the lymphatic capillaries is that they are made of a large number of overlapped lymphatic endothelial cells. An increase in the interstitial fluid pressure causes opening of intercellular floodgates, allowing easy movement of fluid and particles into the interior of these micro-pipes. Once the fluid enters the lumen, the pressure difference across the walls of conduits decrease and floodgates begin to close, thus preventing the return flow into the interstitium [10].

The endothelial cells of lymphatic capillaries are linked to interstitial collagen by anchoring filaments composed of elastic fibers, which preserve the functionality of the lymphatic vessels when interstitial pressure increases, thus preventing total vascular collapse [11]. After lymph formation, this biological fluid drains from capillaries to collectors, which contain one-way valves to help the propulsion of the lymph, as the lymphatic system lacks a central pump. All collector vessels pass through lymph nodes, which are bean-shape structures arranged in clusters along the lymphatic routes.

There are hundreds of lymph nodes in a human adult. They also feature multiple lymphatic capillaries and venules along with blood vessels in close association, thus allowing the exchange of fluid and transportation of cells between the two compartments [12]. The lymph nodes act as filters and reservoirs, and are natural incubators of specialized immune cells and sometimes of tumor cells, which proliferate and then reach the bloodstream. The lymphatic trunks are longer vessels through which lymph flows from the final group of lymph nodes toward the ducts. The exceptions are the lymphatic trunks in the intestinal, liver and lumbar areas which drain directly to a sack-shaped reservoir close to the diaphragm (Chily´s cistern). The thoracic duct is the final route for all lymphatic vessels and it drains it mostly empties into the left subclavian vein, although anatomical variations may occur [7].

TUMORS AND LYMPHATIC METASTASES

The lymphatic net has many advantages over blood circulation as a transportation route for tumor cells. The lymphatic capillaries are much wider, and flow speeds markedly lower, thus maintaining optimal cell viability. Lymph is very similar to interstitial fluid and thus promotes cell integrity [13]. In blood these cells suffer immune toxicity, mechanical deformation and a high shear effect, leading to lower success rates for metastases [14] (Fig. 1).

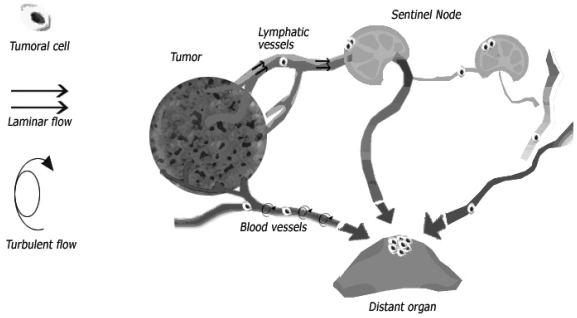

Figure 1. Lymphatic and blood metastases (Modified from reference [48]).

Invasion of lymph nodes is one of the most important prognosis indicators in patients. According to the theory of the SLN, the closest unit is the first to receive lymphatic drainage. Absence of tumor cells in this first location predicts that the following nodes of the lymphatic torrent are probably also free of metastases [15].

Obviously not all tumors metastasize to lymph nodes. Moreover, lymph vessels are not necessarily static and passive throughways for tumor cells. New lymphatic vessels (lymphangiogenesis) may be expressed in the tumor periphery of several human malignancies [16].

The first proteins identified as stimulators of lymphangiogenesis were vascular endothelial growth factors (VEGF) C and D [17]. Both family members of VEGF and the receptor VEGFR-3 induce migration and proliferation of endothelial lymphatic cells, although other markers have been identified such as VYVE-1, podoplanin, and the transcription factor Prox 1 [18]. Overexpression of VEGF D correlates with positive SLN in many cancer lines. Pro-inflammatory cytokines, interstitial pressure and the inherent properties of tumor cells can also affect the speed and routes of metastases [19].

Mannose receptors, which may help the adhesion of malignant cells to the lymphatic system and also interfere with trafficking of these cells are relevant in this context. They have been targeted in the design of new anti-cancer therapies and in the development of diagnostic techniques [20].

USE OF RADIOPHARMACEUTICALS

Besides skin melanoma and breast cancer, multiple invasive lesions of the gastrointestinal, gynecological and genitourinary tract are being currently scrutinized from SLN vantage point [21]. The sentinel screening modality avoids unnecessary regional node dissection, reducing surgical morbidity and mortality [22].

A primitive investigation based on node palpation only, as well as the expression "sentinel node", was applied in penis cancer by Cabanas more than 30 years ago [23]. In the late 80's and early 90's, Morton *et al.* [24] developed peritumoral injection of isosulfan blue dye. In this modality nodes with blue dyed are identified, assessed and removed. As migration of the dye is not fully stepwise, second- or third-order nodes may be stained as well, which limited the reliability of the method. In 1993, lymphatic mapping by radiotracers started with a sulfur colloidal particle marked with technetium 99(^{99}Tc). Similarly to blue-colored nodes, the radioactive structures could thus be recognized. At present the merger of these two methods is the preferred approach for the detection of SLN [25].

A considerable number of radio-labeled molecules are used in medical practice, with a predominance of ^{99}Tc-colloids. The choice of ^{99}Tc is due to its short physical half-life (6 hours), therefore avoiding prolonged contamination of the organism, its high gamma radiation energy of 140 keV which is suitable for imaging, its wide availability and low price [26]. Table 1 lists various radio-molecules appropriate for this purpose [6,27].

Radiolabeled molecule	Particle size (nm)
^{99}Tc-human serum albumin	2-3
^{99}Tc-Dextran	4-5
^{99}Tc-antimony trisulfide	3-30
^{99}Tc- albumin nanocolloidal	5-80
^{99}Tc-rhenium colloid	60-80
^{99}Tc-sulfur colloid	100-400
^{99}Tc-tin colloid	50-1500
^{99}Tc-stannous phytate	150-1500

Table 1. Radio-molecules used in the detection of sentinel lymph nodes and their respective particle sizes [6,30].

An ideal radiopharmaceutical for SLN identification must exhibit maximum uptake by the sentinel lymph nodes, low retention at the administration site and minimum distribution to secondary lymph nodes [28]. In order to achieve such properties it is very important to control particle size. Too small particles, less than 4-5 nm, have the ability to penetrate blood capillaries and will hardly migrate toward lymphatic vessels [29]. On the other hand particles larger than 400 nm have limited clearance from the injection site in the tumor mass [30]. The prompt mobilization of radioactive particles is essential because if uptake by the SLN is low, the local scatter and background radioactivity of the injection site may mask SLNs that are often a few centimeters only from the cancer [31].

Surface charge density is similarly relevant for speed of distribution as electrostatic barriers are operative during transportation through the endothelium of the lymphatic channels [32].

A universal radiopharmaceutical for sentinel node location based on particle size probably will never be developed, because lymphatic irrigation varies among different organs [33]. In case of abundant flow larger particles are preferable, as smaller ones would quickly be carried away beyond the SLN. On the other hand smaller particles are suitable for tumors located in organs with scant lymphatic flow [6]. Table 2 illustrates the range of particle sizes suggested for some types of cancers.

Cancer	Range or particle size (nm)
Skin melanome	200-300
Breast	200-400
Gastric	100

Table 2. Ranges of particle sizes suitable for some types of cancers [25,33].

An easy labeling protocol is of course quite desirable. Nanocolloid of human serum albumin-^{99}Tc is praised for instantaneous labeling at room temperature, although chemical and biological stability should not be overlooked [30].

EFFICACY AND SAFETY

In general, the efficacy of a radiopharmaceutical depends not only on physical, chemical and biological properties of the selected agent,

but also on definition of injection site, volume and dose. Just for breast cancer seven different injection sites are available: intratumoral, peritumoral, subtumoral, subdermal, periareolar, intradermal and subareolar, with the first two the most popular. Some protocols adopt a single technique, others use combinations of these. A few of the mentioned modalities seem to be better suited for recognition of the internal mammary chain and other extra-axillary nodes [34].

Volume (at least 4 mL) and dose of radiocolloid (1-10 mCi) are other questionable contentions. The purpose of large volumes is to increase local pressure, thus forcing lymph flow toward nodes. The rationale behind a high dose is that only a minimal fraction of marker is usually drained to the outside. Pitfalls exist because large volumes may alter physiology of lymphatic drainage [30]. At least for breast cancer, significant differences between peritumoral and subdermal injections are uncommon [35] In both cases the injected volume and applied dose tend to be lower than for intratumoral administration [36].

For melanoma, in the light of the fact that melanocytes grow between dermis and epidermis, intradermal or subdermal inoculation is the preferred route [27]. Three locations are announced for endometrial cancer, namely uterine body, cervix and endometrium. No consensus yet exists on optimal site or number of injections [37].

With regard to gastric cancer, where experience is not vast, 3 mCi of colloidal tin was injected into four points of the submucosa, in the tumor periphery, demonstrating satisfactory distribution [38]. As a general rule it can be affirmed that suitable injection sites are found at the periphery of most tumors, and that doses are comparatively low. This is an advantage as radiation exposure is minimal for the patient and health personnel [39].

ANIMAL MODELS

Animal studies with radiomolecules used in lymph node detection have been mainly carried out in tumor-free mice [31], rabbits [40] and pigs [41]. Lymphatic metastases are nor easy to reproduce in such models, and their size, particularly in small rodents, might preclude conventional manipulation, requiring expensive surgical microscopes and surgical equipment for recognition and surgical removal. Therefore, with the exception of pigs, just general pharmacology and affinity with lymphatic tissue are the target of the investigations. In this sense, most of the protocols are based on injection in foot pads of the front and / or rear limbs to determine the following parameters: a) the time to reach axillary and popliteal nodes, through dynamic imaging studies, b) uptake and retention percentages in the same nodes (Fig. 2) and c) clearance of the radiomolecule from the injection site by bio-distribution studies at different times.

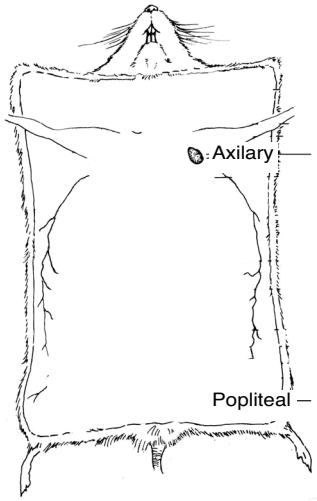

Figure 2. Lymph nodes used in animal models (Modified from www.eulep.pdn.cam.ac.uk/Necropsy_of_the_Mouse/printable.php).

Often the aim is to compare different radiopharmaceuticals and make inferences for human behavior, based on knowledge of radiodrugs already used in medical practice. Investigations correlating results in different species of animals with those of humans, as well as complete clinico-surgical models, are rare. It is worth mentioning that as this is a new medical field, many molecules have not been approved by drug regulatory authorities for this purpose [40].

NEW RADIOPHARMACEUTICALS AND PROCEDURES

The current watchword in drug development is cell specificity, not just particle size as hitherto emphasized. In other words, selective and precise uptake has to be enhanced by differential accessibility [42], thus shifting the paradigm from physico-chemical identification to molecular diagnosis. To achieve this objective, research groups are focusing on molecular markers of lymphatic endothelial cells both in physiological conditions and in the presence tumor-induced anomalies [31,40].

This is easier said than done, because molecular targeting often requires enormous teamwork and huge investments. In the meantime,

existing tools are constantly refined, with the purpose of optimizing clinical diagnosis.

A possible technique based on principles to some extent similar to sentinel node mapping is immunolymphoscintigraphy [43]. Employed in the preoperative phase, it helps in the staging of known lymph nodes already scheduled for lymphadenectomy. In the postoperative period, after the main mass has been removed, its objective is to track remaining nodes in the surgical field as well more remote metastases, although the method is not fully predictable. More promising alternatives are the association of SPECT/CT to lymphoscintigraphy [44], the employment of standard FDG PET/CT or PET/CT with different ^{18}F molecules or timing [45], as well as special software for FDG PET/CT fusion, instead of side by side FDG PET/CT [46].

Functional lymphatic imaging is another route for quantifying lymph velocity and frequencies of propulsion, resulting from the contractility of lymphatic structures. Although currently performed by means of non-isotopic materials, especially fluorescent optical imaging, the perspective exists for radiomarkers as well [47].

CONCLUSION

Current radio-pharmaceuticals employed for sentinel lymph node detection, most of them ^{99}Tc- labeled agents, are designed according to size and other traditional features, but novel approaches are being actively sought. In parallel, the introduction of high-technology functional modalities such as PET/CT and SPECT will be associated with a new array of agents. The molecular, anatomical and physiological knowledge of lymphatic endothelium is therefore defining a new generation of chemical entities, more specific and selective for this pharmacological action.

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